3D Presentation of the Nuclear Cell Features in Quantitative Cytometry

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The use of an image cytometer in analysis of smears and needle aspirates provides valuable information to a cytologist. It allows to combine the overall impression, formed by visually inspecting the cells, with measured and numerically expressed nuclear cell features. Both types of information can be used efficiently only if presented to the expert in an appropriate way. Cell images (as they are seen with a microscope) are easily analysed by the experts. However, measured nuclear features can not be presented as a list of numerical values. Instead, an user interface should be developed, providing graphical presentation of the nuclear features. It should show as much information as possible and provide a comprehensive link between nuclear features and cell images.

The user interface described in this paper shows nuclear features in three dimensions. It is based on a perspective projection of the three dimensional feature space onto a two dimensional surface. It allows the user to dynamically change the perspective, i.e., to look at the virtual three dimensional structure from different viewpoints. Each nucleus is represented by a single object in the three dimensional space. When an object in the three dimensional feature space is selected, the image (or the visual appearance) of the corresponding cell is shown. When a nucleus image is selected, its position in the feature space is highlighted. This provides an interconnection between nuclear cell features and cell images allowing simultaneous analysis of both types of information.

INTRODUCTION

The quantitative image cytometer is an instrument gaining wide acceptance in pathology and cytopathology providing objective measurements of the nuclear cell features[2]. The principal advantage of image cytometry is that high resolution spatial and photometric information is available in the images of microscope fields.

Image cytometer performs cell nuclei analyses, based on the visual appearance of cells stained by a DNA stain (e.g. thionin). Stained cells are mounted on a slide and put under an optical microscope[4,5]. The microscope is equipped with a CCD camera and a computer controlled stage. The CCD camera allows the computer to capture the cell images, while the controlled stage allows it to move the slide and automatically focus and analyse different parts of the slide. After cell images are captured by the camera, they are transformed into digital signals and transferred to the computer. The computer segments the image (i.e. isolates every single cell nucleus), rejecting all objects that are not recognised as cell nuclei. For each nucleus a number of nuclear cell features (for ex. area, DNA amount, sphericity etc.) are calculated. The image and the calculated features are saved for later inspection and classification. Since the camera and the microscope stage are fully controlled by the computer, the cell acquisition process is repeated until enough cells are collected or the entire slide is analysed.

After terminating the cell acquisition process, the cells (or the nuclei) are classified in predefined groups according to calculated features. The classification strategy and the number of groups depend on the type of clinical analyses that are being performed. In cancer research studies, cells are usually classified according to their malignancy. An example of the possible cell grouping would be: normal, CIN1, CIN2, and CIN3. After the cell classification process, results can be summarised to give an overall slide classification and diagnosis.

Cell classification and slide classification can be performed automatically or by an expert. The computer classifies the cells based on the calculated nuclear features, while the expert relies more on cell images. It is obvious that the best classification can be achieved by combining both methods. Usage of both methods is especially important in the phase of development of new classification algorithms and in cancer research, where complexity, speed and cost are not as important as achievement of the best

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possible results. It is hardly possible that computers would ever be able to understand images in the way human experts do, and humans will never be as fast and as accurate as computers when calculation and measurement is considered. A simple but effective way to combine the two approaches is all that a good user interface should provide.

NUCLEAR FEATURES

Nuclear features are numeric values that describe the visual appearance of the nucleus. Some nuclear features (like area of the nucleus, DNA amount or contrast) are self explaining and directly related to parameters that are visually examined by experts. Others (like entropy, homogeneity, fractal area) are complex and in some cases they describe fine changes in the structure of the cell nucleus that are not visible by eye[6]. Nuclear features can be divided in three main groups: morphological, photometric and texture features. In normal cells, changes in the chromatin appearance reflect changes in the activation patterns of genes while in tumours dramatic changes can be seen with the progression of the disease[2,3]. Features describing the chromatin distribution pattern, refereed to as texture features, are sensitive to the differences between the chromatin distribution patterns. A rough division of more than 100 routinely calculated texture features can be summarised by the following categories: 1) descriptive statistics of chromatin distribution, 2) discrete texture features, 3) range extreme, 4) markovian texture features, 5) run length, and 6) fractal texture features.

CELL CLASSIFICATION

A clinical request to separate the cells according to the disease progression demanded application of various classification procedures. Discriminant function analysis, decision trees, statistical classifiers and neural networks are used to distinguish the cells using their nuclear feature values[1]. Statistical classification is based on covariance matrices and discriminant scores or probabilities. Binary decision trees trace classification paths starting with the most informative feature placed in the root, and leading to the leaves (classes). In both the cases, a structure of classification rules can be read. Neural networks realise the classification with back propagation through multilayered structure that remains hidden. All classification procedures consist of two parts: training and classification. In the training process the classification parameters are calculated based on the learning set of data, that was already classified by experts. In the classification process, new data are automatically classified based on previously calculated parameters.

3D PRESENTATION OF NUCLEAR FEATURES

Nuclear features are usually presented in histograms and scatter plots. A histogram shows the approximate probability distribution for a single feature, while a scatter plot graphically shows the value of two features for each cell. These scatter plots are usually called two dimensional feature graphs. In such plots, each cell is represented by a single point. The two coordinates of the point correspond to values of the two presented features. The colour of the point represents the group, that the cell was assigned to during the classification process.

A new graphical presentation described in this paper is an extension of two dimensional scatter plots into third dimension. It shows three features for each cell on a single graph. Each cell is represented by an object (typically sphere) in the three dimensional space. The three coordinates of the object correspond

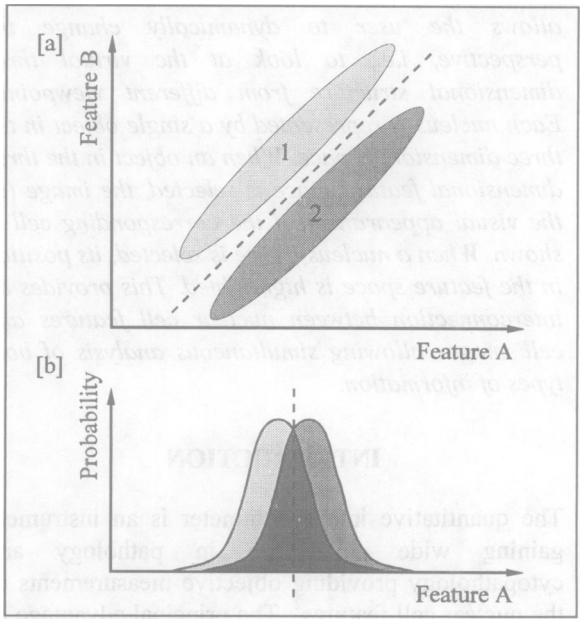

Figure 1 - When features A and B are presented simultaneously [a], it can be easily seen that there are two completely separable groups of data. If only feature A is presented [b], the two groups overlap and they can not be identified or separated.

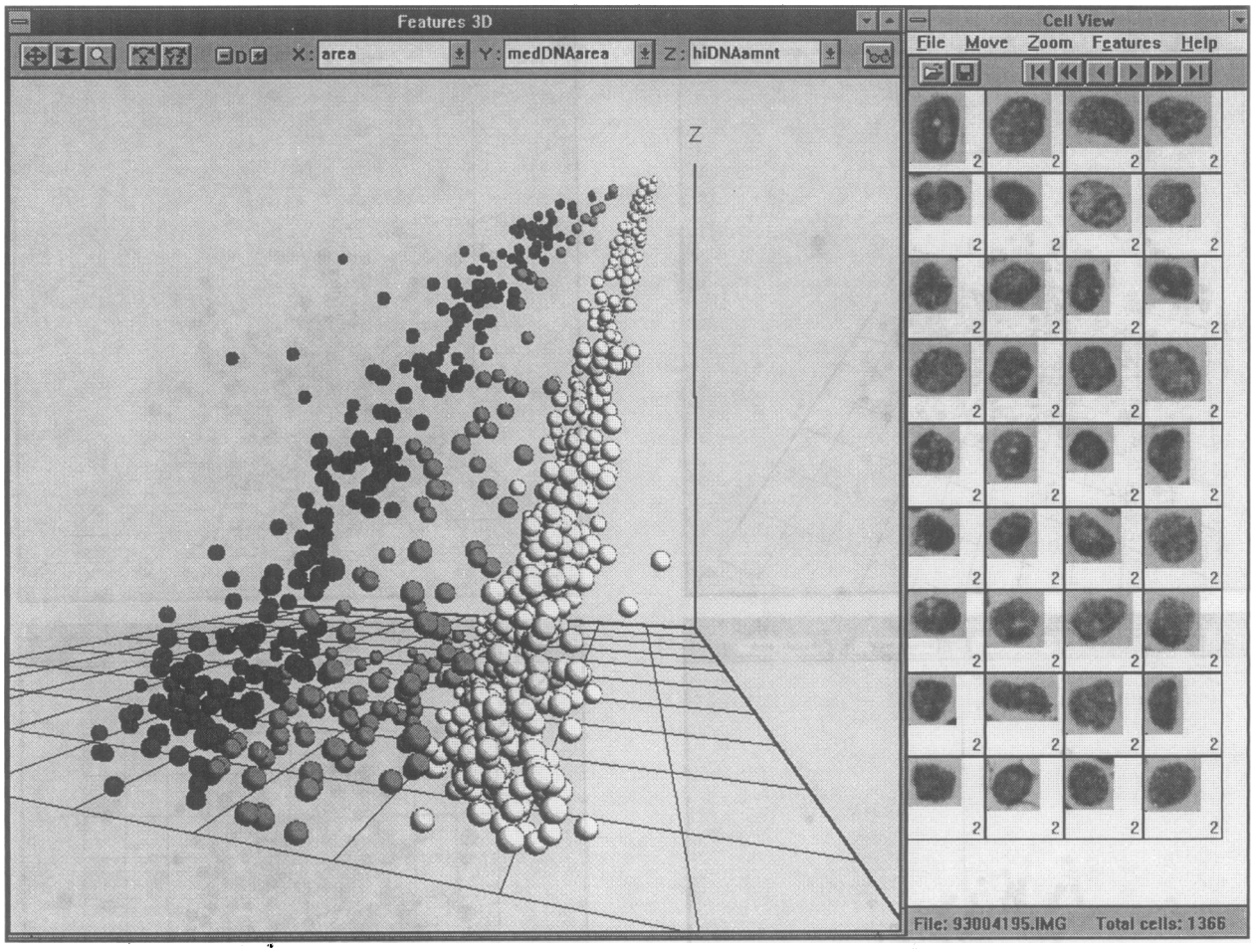

Figure 2 - The user interface shows features as spheres in three dimensions (left window) as well as nuclei images (right window). The two representations are interconnected. When an object (nucleus image or sphere) in one window is selected, the corresponding object in the other window is highlighted.

to values of three presented features. The colour of the object identifies the group the cell is belonging to. Since the computer screen is flat (or two dimensional) it is impossible to show the features in three spatial dimensions on the screen. The described approach is based on a perspective projection of three dimensional feature space onto a two dimensional surface.

This user interface allows the user to dynamically change the perspective and examine three dimensional data from different viewpoints. It is possible to move and rotate the three dimensional coordinate system with plotted data. Nuclear features that corresponds to the axis of the coordinate system can be selected for each axis independently. To increase a three dimensional appearance, spheres, representing the cells that are closer to the viewer are larger than those that are more distant. Spheres can also be shaded as when illuminated by side-light.

The three dimensional scatter plots give more information compared to classical two dimensional scatter plots. In data classification process and clustering analyses, there are various situations when data (or in this case nuclear features) can be distributed in such a way, that when observed in two dimensions, no obvious groups or clusters or any distribution patterns can be seen, no matter which two features are observed. Having the possibility to observe the same data in three dimensions, the expert can better understand how data are structured, and the chances to discover groups of data are much higher.

Scatter plots are seldomly used to classify data. They are mainly used to analyse the results of a

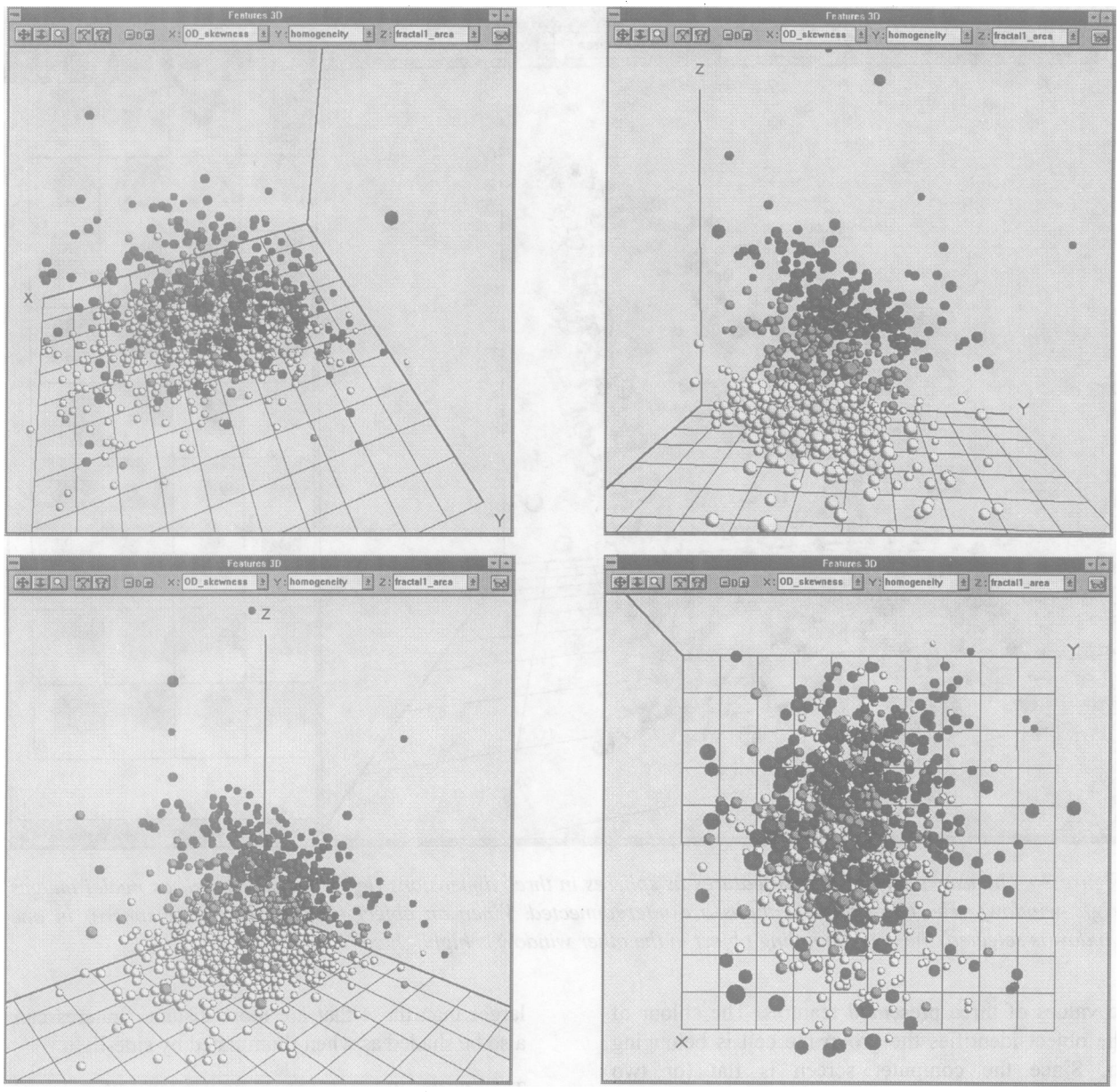

Figure 3 - The same nuclear features represented in three dimensional coordinate system with different perspectives. The top-right window shows the perspective in which the groups of nuclei are visible.

classification or clustering algorithms or to visually confirm some other statistically generated results. If we use, for example, a statistical feature selection method to select three out of 50 nuclear features that give best differentiation between normal and cancerous cells, using a 3D scatter plot, we can inspect the result of the algorithm. We can not only visualise the three nuclear features selected by the statistical algorithm, but we can also draw new conclusions based on position and structure of groups of nuclear features for normal and cancerous cells.

Along with a three dimensional representation of nuclear features, the user interface offers a possibility to link the nuclei images to the graphic representation of their features. When an user selects the image of a nucleus, the corresponding sphere in the three dimensional feature space is highlighted and when a sphere in 3D feature space is selected the nucleus image corresponding to the selected sphere is shown. This enables the user to get a better

understanding of the nuclear features and to directly combine the visual information with automatically calculated data.

The application is written in programming language C. It runs on a personal computer under Microsoft Windows operating system and requires a minimum of 8MB of memory and a 80486 processor. A 17" monitor with a minimum resolution of 1024x786 pixels is recommended. The nuclei images and features used for the development of the user interface were scanned on a Cyto-Savant image cytometer produced by Xillix Technologies, Vancouver, B.C., Canada.

CONCLUSIONS AND FURTHER RESEARCH

The presented user interface for 3D nuclear feature representation provides additional functionality to the cell image cytometer. As the machine enables calculation of the texture feature set, the user interface provides an attractive and simple 3D presentation. Two main advantages appear as a result. First, new relationships between features as well as relationships between features and cell images became visible. A user could easily move from a cell image to its 3D parametric representation, and opposite. Second, two ways of thinking complemented each other: a visual examination relying on the expert's clinical impression and automatically acquired detailed information.

Further research concerns two aspects. From the applicational side, different data classification and clustering methods should be tested and included in the application. Additionally, multiple 3D feature presentation should be developed, so as to allow simultaneous examination of different triples of features, or simultaneous examination of the same nuclear feature triples, classified using different classification methods or training data sets.

From the clinical point of view, knowledge, specific for selected patient groups, malignant changes, cell reproduction cycle should be expressed in terms of cell features. Pointing out and presenting most relevant information could support establishing clinical decisions and a choice of medical treatments.

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